RESEARCH ARTICLE

INHIBITION OF ISPD ENZYME TO CONTROL THE GROWTH OF *MYCOBACTERIUM TUBERCULOSIS.*

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Abstract

Like several other pathogens, *Mycobacterium tuberculosis* synthesizes isopentenyl diphosphate via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. IspD (2C-methyl-D-erythritol-4-phosphate Cytidyltransferase) is a flexible enzyme that carries out the third step in MEP pathway where 2-C-methyl-D-erythritol 4 phosphate (MEP) condenses with Cytidine Triphosphate (CTP) to form 4-diphosphocytidyl-2-amethyl-D-erythritol (CDP-ME). It catalyzes the transfer of a cytidyl group from CTP to methylerythritol phosphate and utilizes two substrates, MEP and CTP. Due to this feature, this stage of MEP pathway is a significant reaction step that can be targeted to control the spread of the organism *in vivo*. Rosuvastatin successfully docks as a ligand close to the active site of the IspD with involvement of low (negative) energy indicating a stable system and a likely binding interaction. It can, thus, be safely assumed that rosuvastatin molecule could possibly be used as a candidate to competitively inhibit IspD to bind to CTP blocking the MEP pathway and keep the proliferating *Mycobacterium tuberculosis* under control.

Introduction:-

Tuberculosis (TB), caused by the bacterial pathogen *Mycobacterium tuberculosis* is one of the oldest recorded human ailments and one of the biggest killers among the infectious diseases (Smith, 2003). New vaccines and drugs are needed to control its worldwide epidemic which assumingly kills two million people each year. To develop new anti-tubercular drug candidates, it is essential to understand the genetics and physiology of *M. tuberculosis* along with its interaction with the host. Search for new molecules that can check the growth and spread of the organism at some early stage of its development is the need of the time. It is urgently required that organism's metabolic targets are identified for novel therapeutics (Brown *et al.*, 2010).

Currently used antibiotics are mostly known to target cell wall biosynthesis in the bacterium. Drugs like isoniazid and ethionamide target the synthesis of mycolic acids and those as ethambutol target arabinogalactan and lipoarabinomannan. A good target might be an enzyme of the Isoprenoid biosynthesis that the organism essentially requires to produce several cellular components including those involved in cell wall formation.

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M. tuberculosis synthesizes the isoprenoid precursor isopentenyl diphosphate (IPP) *via* 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway (non-mevalonate pathway). Targeting an enzyme of MEP pathway would leave the human mevalonate pathway alone sparing us from the side effects of the drug.

The non-mevaslonate pathway starts with condensation of pyruvate and glyceraldehyde-3-phosphate which yields 1-deoxy-D-xylulose 5-phosphate (DOXP). DOXP is converted to 2-C-methyl-D-erythritol 4-phosphate (MEP) which after condensation with CTP forms 4-diphosphocytidyl-2-amethyl-D-erythritol (CDPME) in stage III. Then CDPME is phosphorylated to 4-diphosphocytidyl-2-amethyl-D-erythritol 2-phosphate (CDPME2P) and to 2-C-methyl-D-erythritol-2,4 cyclodiphosphate (ME2,4cPP) which is the last known intermediate of the DOXP/MEP pathway (Wanke *et al*., 2001).

Stage III of the pathway is executed by the enzyme 4-Diphosphocytidyl-2*C*-methyl-D-erythritol Cytidyltransferase (IspD) which is a homodimer. Each subunit of the dimer is a single domain α/β structure constructed around a seven-stranded twisted β-sheet into which is inserted an extended "β-arm." Two arms associate to form the dimer interface that involves numerous hydrogen bonds and salt bridges (Fig. 1).

The active site is created at the dimer interface by seven polypeptide segments, six from one subunit and one from the partner (Hunter, 2007).
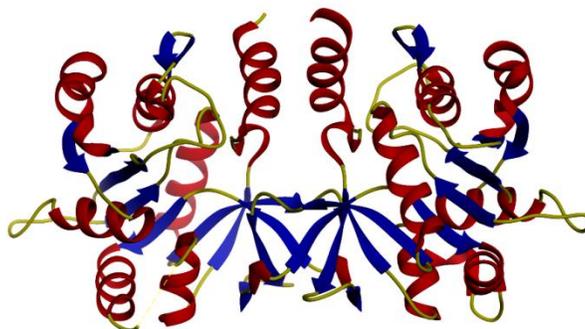

Fig.1:- 3D structure of the dimer IspD.

Materials and Methods:-

3D structural coordinates of 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (IspD) of *Mycobacterium tuberculosis* complexed with CTP was downloaded from www.rcsb.org/pdb with the assigned pdb code 3Q7U (Reddy *et al*., 2011). UCSF Chimera (Pettersen *et al*., 2004) was used to model the structure, remove a chain of the dimer along with the CTP and prepare Chain A for docking with rosuvastatin. 3D coordinates of rosuvastatin (SDF file) was downloaded from PubChem (CID: 446157) and converted to Mol2 file by UCSF Chimera. Docking was set online on SwissDock, a protein-small molecule docking web service based on EADock DSS (Grosdidier *et al*., 2011).

Results and Discussion:-

The calculations in SwissDock are performed in the CHARMM force field. When using default parameters, the whole target protein structure is considered during the docking. The performance of EADock DSS depends on the number of free dihedral angles of the ligand (Grosdidier *et al*., 2011). The Rosuvastatin ($C_{22}H_{28}FN_3O_6S$) comprises 10 rotatable bond count having molecular weight 481.539 g/mol with 3 hydrogen bond donor count and 10 hydrogen bond acceptor count. EADock DSS's algorithm generates upto 15,000 binding modes in the vicinity of the target cavities of the protein surface (blind docking) estimating their CHARMM energies on a grid. Binding Modes (BMs) with the most favorable energies are ranked, taking account of the solvent effect using the FACTS implicit solvation model, and clustered. Finally, the most favorable clusters are presented into the result file (Grosdidier *et al*., 2011).
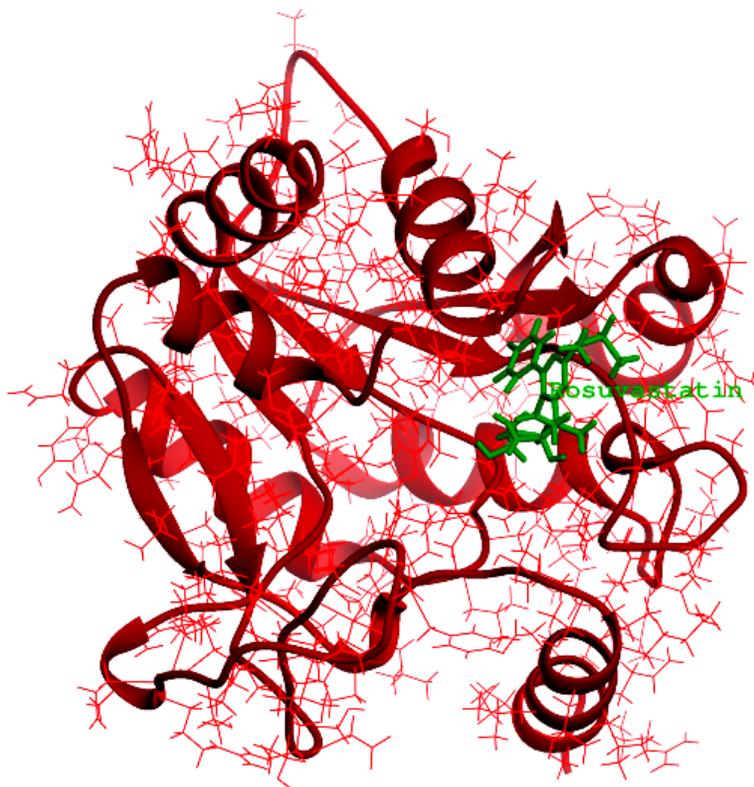
Fig. 2:- Chain A of IspD docked with ligand (Rosuvastatin)
Cluster 19 (Cluster rank 1).
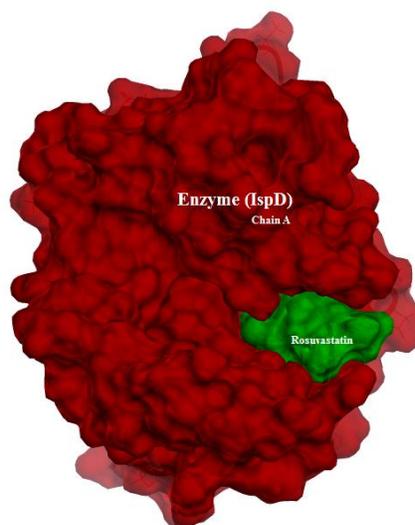
Fig. 3:- Surface view of the ligand docked into the IspD at lowest energy.
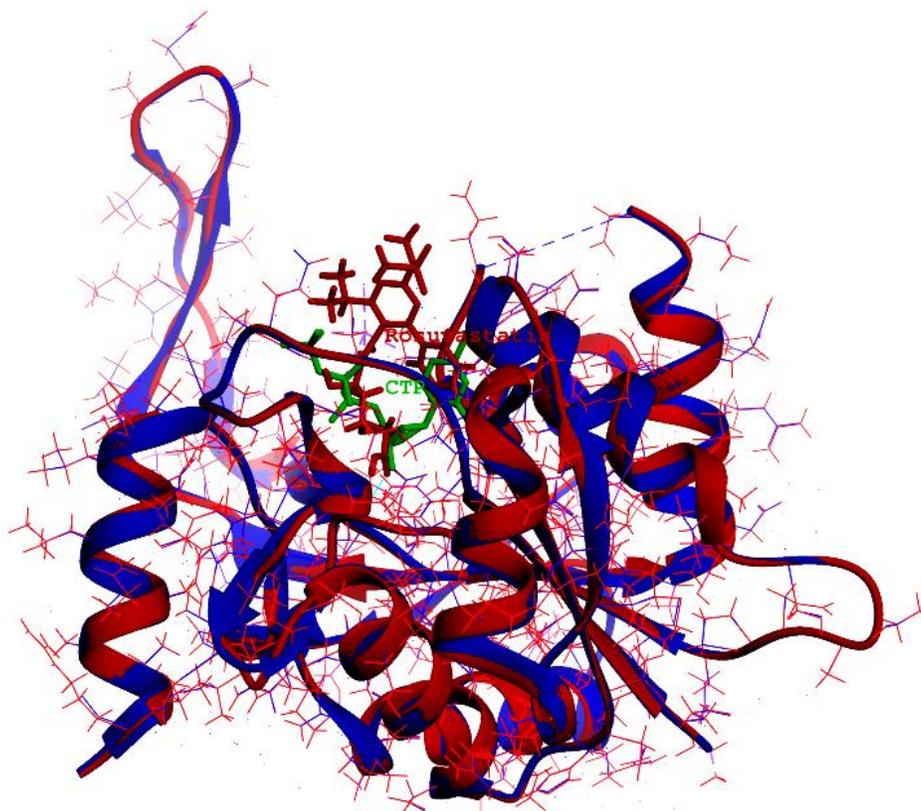
Fig. 4:- Chain A (red) of IspD with the ligand superposed with the crystal structure 3Q7U Chain A (blue) complexed with CTP

Cluster 19 of SwissDock output ranked 1 predicted the best docking mode/binding mode with Energy -0.158562, SimpleFitness -0.158562, FullFitness -1413.9502, InterFull -57.3238, IntraFull -66.3874, solvFull -1463.47, surfFull 173.231, extraFull 0.0, deltaGcompsolvpol -1463.47, deltaGcompsolvnonpol 173.231, deltaGprotsolvpol -1491.03, deltaGprotsolvnonpol 174.309, deltaGligsolvpol -21.7472, deltaGligsolvnonpol 10.994, deltaGvdw -57.3238, deltaGelec 0.0 and deltaG -7.537239. Figs. 2 and 3 depict the rendered structure of the best ligand pose in ribbon and surface views respectively. Fig. 4 presents the superposition of the crystal structure of Chain A of IspD complexed with the CTP (Reddy *et al*., 2011). Björkelid *et al.* (2011) had established the existence of potential flexibility in areas close to the active site in mycobacterial IspD..

The best binding mode of rosuvastatin with the Chain A of the enzyme is almost exactly where CTP binds. It helps to assume that rosuvastatin would bind more reliably and prevent the CTP from binding at the dimer interface where chain A contributes six polypeptide segments. The observation, however, needs support from *in vitro* tests. Varikoti *et al*. (2012) attempted structure based *de novo* designed possible inhibitor molecules for docking into the active pocket of IspD enzyme. Docking of the top hit virtual compound with docking score of -7.559 into the active site showed binding to the hinge region by forming H-bonds with the peptide backbone of Arg20 and Lys215. Obiol-Pardo *et al.* (2010) modelled IspD of *Mycobacterium tuberculosis* to derive a reliable model for use in structure based drug design and they proposed that electrostatic interactions might result in higher affinity of a ligand in comparison of the CTP.

In case of *Plasmodium*, Price *et al.* (2016) could identify 2-phenyl benzo[*d*]isothiazol-3(2*H*)-ones as species-selective inhibitors of IspD. In culture, these compounds displayed nanomolar inhibitory activity preventing the growth of *P. falciparum*. Imlay *et al.* (2015) found that *Plasmodium* IspD could also be an easy intracellular target of MMV008138.

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